THE RELATION OF THE ADRENAL GLANDS TO THE GONADS IN DOMESTIC CHICKENS

by

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INTRODUCTION

The adreno-genital syndrome, a well known, but not common phenomenon in the field of endocrine therapy, led to the view that a similar condition could be produced in experimental animals by injection of adrenal cortex extract. The experimental work which has been done on the relation of the adrenal cortex to the development of the primary and secondary sex characteristics is not conclusive; some workers report results which apparently show a very definite relationship while others conclude that no effects are observed following the injection of adrenal cortex extract, or removal of the adrenal glands. Perhaps one of the chief factors influencing the results of various workers is the difference in the procedures which they followed in preparing the extract, since it is very evident that the type of substances used to remove a hormone from the gland greatly alters the potency of the extract, in some cases to the point of completely inactivating it.

The purpose of this work is further to clarify the relation of the adrenal glands to the gonads and to establish more evidence that there is a gonadotropic hormone secreted by the cortex of the adrenal glands.

The domestic chicken, because of the prominence of its secondary sex characteristics, was regarded as a very favorable experimental animal for this type of work. An increase or decrease in the size and activity of the gonads is soon manifested in the appearance of the comb and wattles. Because of the close relationship which exists between the primary and secondary sex characteristics, and the prominence of the latter in the chicken, it is possible to make close daily observations, without the necessity of sacrificing the animal. The increase or decrease in size of the comb in fowls is an accurate index to the major changes in the gonads.

That the adrenal glands have an important role in maintaining life and normal body functions has been known since 1855, when Addison discovered the diseases which still bears his name.

Recent experimental work has shown that the adrenal cortex also plays a role in maintaining a balance between the sodium and potassium in the body, and there is considerable evidence that it is important in the utilization of carbohydrates.

Pliny, nearly two thousand years ago described cases of extremely early sexual maturity in children. No account is given which would lead to the view that adrenal malfunc-

tion was the cause of the conditions described. Recently early sexual maturity has been reported several times. Post mortem examination has invariably revealed an abnormal cortex in these cases. On this basis it is logical to suppose that the adrenal cortex was responsible for the conditions described by Pliny.

During the past ten years considerable important work has been done on the relation of the adrenal cortex to the sex organs, and today research on this subject is a very popular pursuit among endocrinologists.

REVIEW OF LITERATURE

Literature on the relation of the adrenal glands to the gonads in fowls is not abundant. Considerable work has, however, been done on this relationship in white rats and mice. It is advisable, therefore, to review briefly some of the literature which definitely points to a relationship between the cortex of the adrenal gland and the activity of the gonads.

The work published on the relation of the adrenal glands to the gonads might well be divided into two main groups, or better two opposing factions:

(1) That work which definitely points to a relationship.

(2) The work which gives evidence for no relationship

existing. The literature on the positive results is considered first.

Britton and Kline (1933) obtained reduced fertility in both male and female rats as a result of adrenalectomy. Injections of cortico-adrenal extract has in some cases induced labor and terminated pregnancy in unoperated animals.

Grollman and Howard (1933) found that the testes of rats injected with cortico-adrenal extract averaged 20 per cent heavier per body weight than in controls. In the female there was no effect on the age at which the vagina opened, and no effect on the estrus cycle was observed.

Brownell, Lockwood, and Hartman (1932) made an extract of the adrenal cortex. This extract when injected into adrenalectomized mother rats caused secretion of milk. Their extract was named cortilactin.

Casida and Hellbaum (1934) made extracts of eight different lots of adrenals from non-pregnant mares and geldings (pyridine extract), and tested it on 25 day old female rats for gonadotropic activity. Each lot of adrenals gave positive ovarian responses, large follicles were formed. Corpora lutea also were found in the ovaries of the females following injection of extract from each lot of adrenals. Ovulation occurred in 50 per cent of responding test animals. The greatest ovarian response (in weight) was ob-

tained from the adrenals of mares during the time their blood showed the greatest gonad-stimulating activity. Positive results were obtained from adrenal extract of non-pregnant mares and geldings, even though the blood of these animals showed no gonad-stimulating activity. This gives evidence that the hormone which they extracted was not a product of the sex organs or the pituitary gland.

Carr (1931) concludes that pregnancy in the white rat does not prolong the survival period when both adrenal glands are removed.

Corey and Britton (1932) injected cortico-adrenal extract into normal rats, and found increased activity in the estrus cycle. Later, in many cases, inhibition of estrus was noted. They used a modified Swingle and Pfiffner extract. Cortico-adrenal extract restored the estrus cycle in adrenalectomized rats in which it had been inhibited for a long period. The same workers (1931) observed precocious maturation of the sex glands of young albino rats following injection of Swingle and Pfiffner extract. Hypertrophy of the uterus and appearance of maturity in the testes occurred later but was not so striking.

Eaton, Insko, Thompson and Chidester (1929) found that chicks fed on desiccated adrenal cortex had larger testes than the controls fed on an ordinary ration.

Hypertrophy of the testes of white rats fed on whole adrenal gland was reported by Hoskins (R. G. and A. D.) (1916).

Vincent (1917) also noted stimulation of testicular growth in young animals fed on adrenal gland substance.

Hewer (1922) found a hastening of genital development in the testes of rats by feeding of adrenal cortex, later, however, there occurred a degeneration of the seminiferous tubules.

That adrenal cortex fed rats had 21.5 per cent heavier testes than the control animals was the result of the work of McKinley and Fisher (1926).

By feeding of cortico-adrenal substance, Chidester, Eaton, and Thompson (1929) obtained a hastening of maturity in rats.

Gaunt and Tobin (1936) observed that four out of 10 adrenalectomized mother rats treated with Swingle and Pfiffner extract raised litters. Two times the adequate amount to keep the mother alive caused normal lactation.

It was observed by Gaunt (1933) that adrenalectomized female rats which bore normal litters,did not lactate adequately to keep the young alive unless accessory adrenal bodies remained.

Carr (1931) injected adrenalectomized mother rats with

1 cc. (40 gm. beef adrenal cortex) daily, beginning on the day of the operation. There was a noticeable decrease in milk on the second day after operation and lactation ceased on the third day. The young died on the fourth day after the operation on the mother. He concluded that Swingle's extract does not contain a lactation hormone.

Connor (1931) gave by injection doses of cortico-adrenal extract (Swingle and Pfiffner) amounting to 20 gm. adrenal cortex to 200 gm. rat weight. The controls matured as early, or earlier than the experimentals. Laying hens injected with 1 cc. every other day, receiving 25 injections in 49 days, continued laying normally, producing an average of 21 eggs during the period. No effect was observed in the estrus cycle in rats receiving 1 cc. of Swingle's extract daily for seven days.

Gaunt and Parkins (1933) injected "Eschatin" intraperitoneally into rats, and subcutaneously into chicks prior to age of sexual maturity (5 to 20 dog units per day) for a long period of time and found no effect on the reproductive system in either the chicks or the rats.

Grollman (1936) gives considerable attention to a special layer in the adrenals of man. To this layer, which is called the androgenic layer, is attributed the condition manifested in the adreno-genital syndrome. It is found in

the human adrenal only during early infancy, undergoing involution with age.

Howard (1927) found in the mouse adrenal an area which she believed to be homologous to the androgenic layer in the human adrenal gland.

That the adrenals of fowls are reduced in size after maturity, even though there is an increase in body weight, was observed by Elliot and Tuckett (1906). This gives some evidence that there is androgenic tissue in the adrenal glands of fowls.

Suppression of the estrus cycle was found by Martin (1931) in 87.6 per cent of female adrenalectomized rats. He also found a change in the cytological picture of the hypophysis of the adrenalectomized rats, and a decrease in the gonad stimulating hormone of the anterior lobe of the pituitary. Subcutaneous injections of theelin restored estrus.

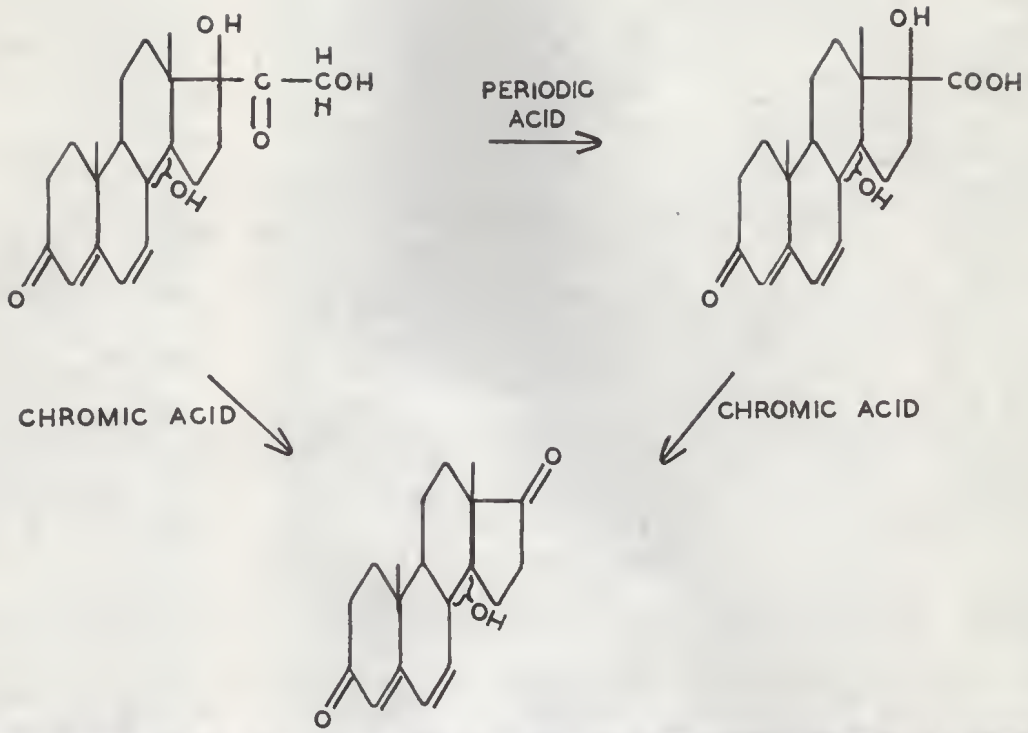
Nice and Shiffer (1931) transplanted adrenal glands from young adult rats, nine to 10 months old, into the dorsal muscles of females which were 16 to 23 days old. The vaginal canal opened in six of the 10 rats which survived adrenal transplants. This occurred six, eight, 10, and 11 days after the transplants were made, the rats ranging from 23 to 28 days of age at the time when the vaginal canal opened.

Riddle (1923) found that the adrenal glands increased approximately 40 per cent in size during ovulation in the pigeon. Adrenal transplants in females seemed to result in smaller ovaries than in control doves, while in males into which adrenal gland was transplanted the testes were larger than those of the controls.

Wyman (1928) claims there is a partial or total inhibition of the estrus cycle in adrenalectomized animals, the inhibition depending upon the degree of insufficiency.

A change in the volume of the cortical tissue during the period of egg production in fowls was observed by Sun and MacOwan (1930).

The work of Mason, Myers, and Kendall (1936) has a very important relationship to the present work on adrenalectomy. A substance which possesses the qualitative action of cortin has been isolated by these men. This substance (known as fraction E) can be converted into a diketone closely related to androstenedione by oxidation with chromic acid. The same ketone results from the treatment of the fraction E with periodic acid and further oxidation of the intermediate acid, formed, by chromic acid.



Koch tested the ketone for comb-growth stimulating activity in capons, and found it to be one-sixth to one-fourth as active as androsterone in this respect.

Reichstein (1936) isolated a crystalline substance from the adrenal cortex. This substance was converted into a ketone, which he called adrenosterone. He found that a daily dose of about 3.5 gamma when dissolved in 0.1 cc. of oil and spread on the comb of capons for four consecutive days, resulted in increased rate of comb-growth.

That the estrus cycle ceased in all but one rat receiving 12 to 15 injections daily, of two cc. watery extract of adrenal cortex was found by Connor (1931). The extract was prepared by macerating adrenal cortex from beeves in an equal amount of Ringer's solution and filtering it through a Seitz filter. Degeneration of the ovaries of laying hens was obtained by injection of the watery extract.

This gives evidence that the adrenal cortex contains a substance inhibiting the activity of the gonads.

Shiffer and Nice (1930) conclude that adrenalectomy does not appreciably affect the estrus cycle.

Jaffe and Marine (1923) observed marked ovarian enlargement in 76 per cent of the rabbits which survived double adrenalectomy over 30 days. The enlargement was essentially a hypertrophy of the interstitial cells. Their view is that the interstitial cells of the testis and ovary are not functionally homologous for no marked change occurs in the interstitial cells or the tubules of the testes in compound adrenalectomized rabbits.

ANATOMY, HISTOLOGY, AND LOCATION

The adrenal glands of the chicken are located between the kidneys and the lungs close against the spine. The inferior vena cava courses close to their lateral margins between the two glands.

A description of the shape of a typical chicken adrenal gland is difficult; in the first place because of differences in the same bird, and secondly, because of variations among different birds. It is usually flattened on its ventral margin and tapers to a blunt point on the dorsal extremity. The shape seems to be modified somewhat,

depending upon the distance between the kidney and the lung, for it occupies a pocket between these two organs.

The adrenal glands of rats, cats, dogs, mice and other mammals, show a definite cortical region surrounding the medulla. In the chicken, however, the gland has a thin protective covering and histological examination reveals no true cortex and medulla, as such, but clusters of cortical and medullary cells are scattered among each other throughout the gland. (Figures 1 and 2)

Latimer and Landwer (1925) projected sectors of every 20th section, of chicken adrenal, on drawing paper. Tracings of the two types of cells (medullary and cortical) were cut and weighed. The medullary cells from male birds averaged 71 per cent of the cortex, the individual percentages were 65 and 79. In females the medulla averaged 37 per cent of the volume of the cortex, a range of 28 to 71 per cent. The cortical cells are grouped in larger masses toward the periphery; toward the center of the gland the cortical cell masses are smaller, while the medullary cell masses are larger.

Sauer and Latimer (1931) gave an account of the shape and arrangement of the cells of the adrenal glands in the chicken. In the outer portion of the gland the cortical cells are arranged in columns and folded layers. The cells

are elongated in this outer region, and their long axes are transverse to the plane of the layer. In the central part of the gland the cortical cells are clumped together. The cells of the medulla have a round, but somewhat irregular shape. There is approximately 30 per cent more cortex (in proportion to the body weight) in the female than in the male. The total weight of the gland is about the same in both males and females. The amount of cortical tissue in the female varies considerably, while in the male the variation is not as great.

Considering comparative sizes, the blood supply of the adrenal gland is the richest of any organ in the body. It is estimated that six times its own weight of blood passes through each gland every minute (Hoskins). The cells of the gland are in intimate contact with large sinusoids, in which blood flows. This insures liberation of the hormones into the blood stream.

MATERIAL AND METHODS

Injection of Cortico-Adrenal Extracts

The conclusions of earlier workers concerning the results of injection of "Eschatin", a Parke-Davis adrenal cortex extract, into rats are conflicting. Because of these confusing results it was deemed advisable to repeat

the above mentioned experiment on fowls. The comb of the chicken is very sensitive to gonad stimulating hormones, and can be observed and measured daily, thus serving as a very close check on the activity of the gonads.

Twelve White Leghorn chicks and an equal number of controls, two days old, were put on experiment January 16, 1936. The extract used was "Eschatin". It is prepared after the method of Pfiffner and Swingle (1931) and is a potent extract, with respect to maintenance of life in adrenalectomized animals. One cc. of the aqueous extract represents 40 gms. of fresh cortical tissue.

The chicks were fed on a ration consisting of: corn meal--38 per cent; ground wheat--38 per cent; dried milk--12 per cent; alfalfa meal--5 per cent; bone meal--5 per cent; and salt--1 per cent. They were kept in the Experiment Station animal house, in brooders provided with 100 watt light bulbs for heat.

The injections were continued from January 16, 1936 to February 27, 1936. The dosage given was 0.1 cc. intraperitoneally. Injections were made on alternate days; first, third, fifth, etc., with a rest period of one day between injections. The birds were weighed weekly. After a period of 41 days the birds were killed and a thorough post mortem examination was made. Special attention was given to the

testes and vas deferens of the males and the ovary and oviducts of the females. A comparative study was made on the size of the gonads of the experimentals and the controls. The gonads, vas deferens, oviducts, and adrenal glands were placed in Bouin's fixative and saved for histological study at a later time.

A comparative post mortem examination of the experimentals and the controls revealed no noticeable change due to the injection. In some cases the gonads of the controls were larger and more mature than those of the experimentals. The experimental birds as well as the controls showed a uniform increase in weight, and no ill effects were observed as a result of the injections.

A comparative histological study of the gonads and the adrenal glands of the experimentals and the controls failed to give any indication of the presence of a gonadotropic hormone in "Escbatin", the Parke-Davis Company's adrenal cortex extract.

Ehrlich's hematoxylin and Eosin were used in staining all tissues studied.

The preceding experiment was not considered as conclusive for two reasons: (1) Because of the small quantity of extract given, and (2) too long intervals between injections. In order to ascertain the presence or absence of

the sex stimulating fraction in the Parke-Davis extract, it was deemed necessary that a larger dose of the extract be given, and that injections be made more frequently.

A typical male and female were selected for this experiment, and one of each sex to serve as controls. One-half cc. of "Eschatin" was injected intraperitoneally daily for a period of 28 days. The birds were weighed daily in order to make a close observation of retardation or acceleration in growth. On the day following the last injection, the birds were killed and examined. The gonads, oviducts, vas deferens, and adrenal glands were placed in Bouin's and fixed for histological study.

Comparative study of the size of the gonads and adrenal glands of the control and the experimental birds gave strong evidence that the gonadotropic hormone of the adrenal cortex is not present in the Parke-Davis extract. Histological study of the organs mentioned above gave further evidence of the inactivity of the extract. There was a uniform increase in weight of both the experimental and the control birds.

The Harrower Laboratory, Inc., has on the market, an extract of the adrenal cortex, known as "Adreno-Cortin". One cc. of this extract represents the amount of water soluble active principle present in five gms. of adrenal cortex. The method of extraction which they followed was dif-

ferent in some respects from the procedure of Pfiffner and Swingle, so it was considered valuable to use their extract to determine whether it contained the sex stimulating fraction.

A typical male and female 2 months of age were selected for the experiment. One cc. of "Adreno-Cortin" was administered intramuscularly, in two doses, daily, for a period of three weeks. The birds were weighed every other day. At the end of three weeks the birds were killed and the adrenal glands and the gonads saved. Post mortem examination and histological comparisons of the reproductive organs and adrenal glands were carried out as previously stated in the experiment on "Eschatin". No gonad stimulating activity was observed as a result of the injection of this extract.

Removal of the Gland

It became more and more evident as the work was continued that the most logical method of studying the relation between the adrenal glands and the gonads would be to remove the adrenals. Only one case of adrenalectomy in birds has been noted in the literature to date.

Gourfein (1896) removed the adrenal glands from pigeons. Cortin was unknown at this early date, so it was not possible to keep the birds alive for more than from five to

24 hours after adrenalectomy. The result of Gourfein's observations, therefore, did not include more than a description of the symptoms of adrenal insufficiency. No study could be made on the relation of the adrenal glands to the gonads in such a short period of time.

The difficulty of removal of the adrenal glands because of hemorrhage has discouraged the use of birds for studying the results of adrenalectomy. The first attempt at removal was the usual method of separating the glands from the surrounding tissue by means of forceps. The incision was made between the last two ribs, extending as far dorsally as possible. The ribs were spread by a spreader particularily designed for this purpose. The glands were easily reached, but in view of the rich blood supply to the adrenals, removal of the glands with forceps proved to be very unsatisfactory. Even though the gland could be pulled away from the post cava and separated from the smaller blood vessel passing across its posterior ventral margin, a fatal hemorrhage would invariably result as the gland was pulled away from its dorsal attachment. Even a puncture of the substance of the gland often led to a severe hemorrhage. After several fruitless trials it was concluded that this method was not satisfactory.

Some of the workers in endocrinology have successfully

used a cautery in destroying glands or parts of glands. Hartman (1933) caused adrenal insufficiency in cats by extensive cauterization of the glands. This method was also used in operations by early surgeons and it decreased the danger of hemorrhage very appreciably. Modern surgeons sometimes employ cautery to prevent bleeding. Because of the success of other workers in reducing hemorrhage by the use of cautery, it was concluded that this method might make possible the removal of the adrenal glands in fowls.

The cautery used was made by connecting a single strand of Nichrome wire to ordinary light cord and insulating the union with asbestos paper. The asbestos was held in place by wrapping with friction tape. Approximately one inch of the loop formed by connecting the ends of the filament to the light cord was left free from asbestos and tape and constituted the cauterizing portion of the instrument. A coil of Nichrome wire approximately 48 inches long was inserted as a resistance between the cautery and a 220 volt circuit. When the resistance was properly adjusted the cauterizing end of the instrument would be red even when in contact with the moist glandular tissue. (Figure 3)

White Leghorn chickens were used in the experiment. Some had reached sexual maturity, while in others the reproductive organs had not, as yet, enlarged.

Food was withheld from the birds from 12 to 15 hours previous to the operation, in order that the stomach and intestine would be void of food. This greatly facilitated reaching the glands and it also appeared to decrease the danger of death from an overdose of the anesthetic.

The glands were removed in two operations; in some cases one week lapsed between the operations, and in one case two weeks. The length of time between the operations is important in maintaining the birds after complete adrenalectomy. Rogoff and Stewart (1926) found that the average life span of their adrenalectomized dogs was seven days. They removed the glands in two operations, under ether anesthesia. About 10 per cent of the animals lived from 10 to 12 days. Grollman, Firor and Grollman (1935),also using dogs as experimental animals, removed the adrenal glands in a single-stage operation under spinal anesthesia. The average survival period was found to be four days. These experiments indicate that it is preferable to remove the adrenal glands in two operations.

The bird was laid on its side on an operating table with adjustable top. The legs were tied to one end of the table and the wings to the other. In this way it was possible to exert sufficient pull on the legs so that the muscle of the thigh would not be in the way at the point of incision.

At first ether was used as an anesthetic, but it was not entirely satisfactory for use on birds because it sometimes failed to give complete relaxation. Another factor in administering ether to a bird is the difficulty of determining the stage of anesthesia. Pentobarbital Sodium, commonly known as Nembutal, was found to have several advantages over ether. When injected intravenously it produces anesthesia and complete relaxation immediately. The effect lasts for about two hours, when a normal dose, of 1 cc. for each five pounds of body weight, is given. It does not affect the respiration as ether does but produces a slow uniform respiratory rate. This fact greatly facilitates work of this type, where entry is made between the ribs. The feathers were plucked from the side of the bird and entry was made between the last two ribs, the incision made to extend as far dorsally as possible. A suitable spreader was used to spread the ribs to provide room for manipulating the cautery. The destruction of the tissue was begun on the mid-ventral portion of the gland, gradually working inward to the center. The periphery of the gland was the last to be cauterized. This procedure was advisable for injury to the major blood vessels could be avoided.

The use of a cautery did not, however, completely remove the hazard due to hemorrhage, for the gland is located

in such a position that there is danger of severe bleeding from three main sources. These sources of hemorrhage are the lung, lying close to the front of the gland, the kidney, behind and laterally, and the post cava, a large sinus-like vessel medially and ventrally. In addition to these, blood has been observed to spurt out of the substance of the gland at the place where it was being cauterized. There is little opportunity to ligate, only one small vein across the ventral side of the gland is in such a position that ligation is possible.

Lysol was used as an antiseptic during the early part of the work, and was very satisfactory except that it forms a soapy solution and makes the instruments slippery to handle. Germicidal discs of Potassio-Mercuric Iodide, a Parke-Davis Company product,was found to be very satisfactory. Chickens are, however, very resistant to infection, so the type of antiseptic is not an important factor. Ordinary cotton thread, number 40, was used in suturing. The ribs were pulled together by three or four stitches and the skin wound closed except for a quarter of an inch which allowed air to escape. If the entire wound was sutured, air puffs would result because the skin wound heals more rapidly than the tissues between the ribs. The stitches inside the body were not removed.

The second adrenal gland was removed a week after the removal of the first, except in one case (previously cited) in which a two-week period lapsed between the two operations. The procedure was the same as in the first gland except that entry was made on the other side of the bird. At the time of removal of the second gland the testis on that side was measured. In this way each bird served as its own control. The same testis was measured when the bird died or was sacrificed. The comb was carefully measured at the time of the second operation, and weekly thereafter.

Complete removal of the adrenal glands results in the death of the bird in from six to 15 hours after the removal of the last gland. For this reason it was impossible to ascertain whether the operation was successful by the method employed on other animals, that is, allowing the birds to become depleted and manifesting the depletion by the typical symptoms of adrenal insufficiency. The only method which could be used on birds was to give injections of adrenal extract and saline solution and make careful records on the size and color of the comb from day to day.

Rubin and Krick (1934) showed that adrenalectomized animals could be kept for a considerable period of time on a solution containing the following salts: .0329 per cent $CaCl_2$, .015 per cent $MgCl_2$, .7 per cent NaCl, and .0350 per cent KCl.

Maintaining the birds after adrenalectomy and preventing loss of body weight was a very important part of this experiment. Since adrenalectomized birds live for only a very short period of time unless adrenal cortex extract is given, it was necessary to begin the administration of extract immediately after the removal of the last gland. Before the bird had recovered from the anesthetic, 0.5 to one cc. of "Eschatin" was given intramuscularly; the injection was made in the muscles of the breast. Injection of one cc. of extract daily, in two doses, was continued for two or three days following the operation. Sodium chloride was added to the drinking water as soon as the birds recovered from the anesthetic and were able to drink. Drinking water containing 16 gm.. of sodium chloride per liter of water was available to the birds at all times. Each bird drank from 1.5 to two liters in 24 hours. The sodium chloride content of this amount was approximately 24 to 32 gm..

The second or third day after the operation, injections of normal saline solution were substituted for the cortical hormone. Twelve to 15 cc. of normal saline were given daily in two or three doses. The saline injection and sodium chloride in the drinking water maintained the body weight, and the birds were active even though they received no cortical extract.

The large quantity of water, as well as the sodium chloride, was probably beneficial in maintaining life, for a considerable amount of water is lost from the body of adrenalectomized birds.

OBSERVATIONS AND RESULTS

Complete adrenalectomy results in the death of the chicken within six to 15 hours. One of the most noticeable symptoms of adrenal insufficiency is weakness, first manifested in the appendages. The birds recover from the effects of the anesthetic in about four hours. They then walk about, eat, drink and appear to be normal. In a few hours they are unable to stand, become drowsy and in a short time death follows accompanied by respiratory difficulty. In birds which were given saline solution and adrenal cortex extract immediately following the removal of the last gland, life was prolonged as long as 82 days. This particular bird was killed at the end of that time. Cortin was given for only two or three days after the operation; the amount of saline given was increased when cortin was withdrawn.

Changes in the comb may appear as early as the second day after adrenalectomy. The comb which normally is erect and full, begins to lose its rigidity and falls over to one side, becoming lighter in color. The comb gradually be-

comes shorter and also decreases in height. The points become narrower and the notches between the points become deeper, so that the comb closely resembles that of a capon in size as well as in general appearance. (Figures 4 and 5)

The neck feathers change to the long silky type characteristic of the capon. The legs increase in length, hence the movement of the bird is clumsy and slow resembling that of a capon.

Post mortem examination revealed a marked change in the size of the testes. In a very short period of time they decreased to a small fraction of their size before adrenalectomy. A comparison of the testes of the experimentals with those of the controls gives even a greater contrast. (Figure 6)

That the testes of the experimental birds are modified considerably in histological structure is revealed by microscopical study of cross sections of these glands. There is a very evident decrease in the size of the interstitial cell masses, as well as in the size of the individual interstitial cells. The tubules of the testes of the experimental birds are very appreciably reduced in size, and modified in structure. The Sertoli cells are separated from the interstitial cells to the extent that spaces are formed between the interstitial cells and the Sertoli cells. No lumina,

as such, are recognizable in the testes of the experimental birds, for the Sertoli cells are massed together in the central portion of the tubule, thus closing the lumen. It was found by Freed, Brownfield and Evans (1931) that the testes of adrenalectomized rats were pale, soft and edematous. The testes of immature adrenalectomized rats were lighter in color than those of the controls. Histological examination of the testes revealed ragged, fragmented, and disorganized tubules. They also found cells in the lumina of the tubules and often found that only the germinative layer remained in position. Spermatocytes stained ghost-like with eosin. The results reported by these men on rats are very similar to the results obtained in the present experiment. Various stages of maturation of sperms were observed in the experimental bird's testes. Sperm cells, however, were limited to widely scattered groups, containing only two or three in each group. The testes of the controls contained a vast number of sperm cells; several hundred were observed in each tubule as cross sections of the testes were studied microscopically. (Figure 7 and 8)

SUMMARY

1. The adrenal cortical extract of the Parke-Davis Company sold under the trade name, "Eschatin", did not

stimulate comb growth or cause precocious development of the gonads in young birds.

2. "Adreno-cortin", a Harrower Laboratory product, gave no evidence of gonadotropic activity.

3. The use of an electrical cauterizing instrument made possible the removal of the adrenal glands in chickens.

4. Removal of the adrenal glands in chickens is followed by death in from six to 15 hours unless cortin, or saline solution supplemented with cortin, is given.

5. Observed symptoms of adrenal insufficiency are asthenia, loss of appetite, diarrhea, and finally respiratory difficulties followed by death.

6. Bilaterally adrenalectomized chickens can be maintained for a long period of time by injection of adrenal cortex extract. Injection of normal saline solution and addition of sodium chloride to the drinking water is also very effective in prolonging life.

7. The chief difficulty in adrenalectomy in the chicken is hemorrhage. It may occur from any of four sources; the lungs, kidneys, post cava or the adrenal glands.

8. The danger of hemorrhage in adrenalectomy, though not completely overcome, is materially reduced by the use of an electric cautery.

9. Adrenalectomy results in a modification of the primary and secondary sex characteristics in the chicken.

10. The histological structure of the testes of adrenalectomized birds is greatly modified. The interstitial cell masses, as well as the individual interstitial cells are appreciably reduced in size. The Sertoli cells are disorganized and come to lie in the lumina of the tubules. Prominent spaces are present between the mass of Sertoli cells, in the lumen, and the interstitial cells. Spermatogenesis is very appreciably retarded by adrenalectomy.

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TABLE AND PLATES

Table I. The changes in the size of the comb and gonads following adrenalectomy.

Bird Number	Age at removal of 2nd gland	First change in comb noted	Survival period	Original size of left testis	Size of left testis at death	Original height of comb	Original length of Comb	Height of comb at death	Length of comb at death
A 56	8.5 mos.	2nd day after operation	14 days	25 mm. long 17 mm. wide	15 mm. long 13 mm. wide	75 mm.	111 mm.	55 mm.	97 mm.
A 232	4 mos.	Several days	53 days	26 mm. long 13 mm. wide	11 mm. long 5 mm. wide	67 mm.	111 mm.	52 mm.	97 mm.
A 300	4 mos.	3rd day after operation	82 days	had not enlarged	10 mm. long 4 mm. wide	54 mm.	81 mm.	33 mm.	54 mm.
A 58	8.5 mos.	2nd day after operation	17 days	24 mm. long 15 mm. wide	17 mm. long 10 mm. wide	66 mm.	112 mm.	64 mm.	94 mm.
A 60	Sexually mature	Several days	32 days	34 mm. long 20 mm. wide	25 mm. long 10.5 mm. wide	72 mm.	133 mm.	68 mm.	126 mm.
A 38	3 mos. 11 days	4th day after operation	35 days	13 mm. long 5 mm. wide	8 mm. long 3 mm. wide	30 mm.	63 mm.	20 mm.	45 mm.

Explanation of Plate I

Figure 1. Photomicrograph of cross section of a rat adrenal gland showing cortex and medulla. A typical mammalian adrenal gland.

Figure 2. Photomicrograph of cross section of a typical chicken adrenal gland showing cortical and medullary cells.

Plate I.

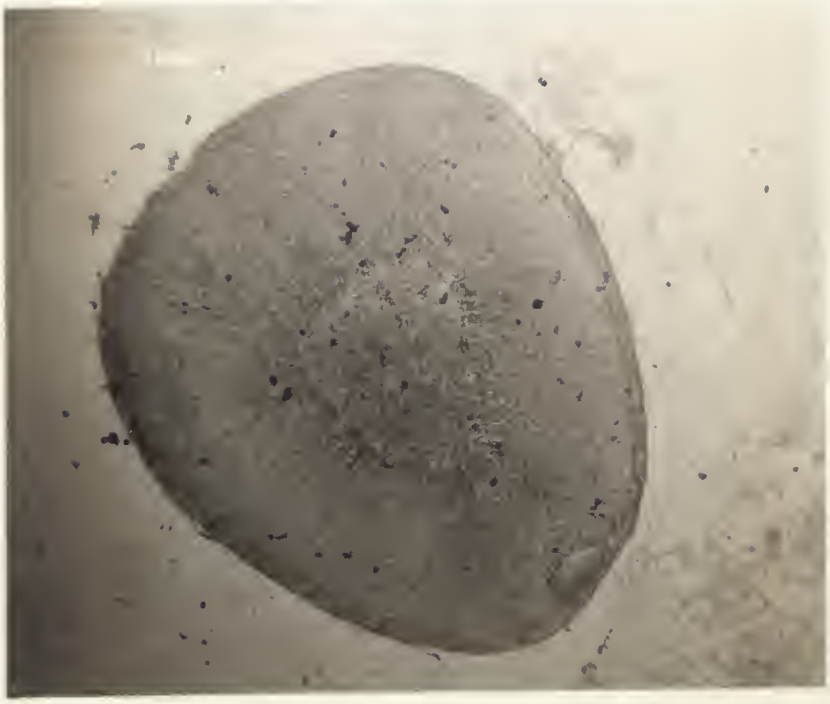

Figure 1.

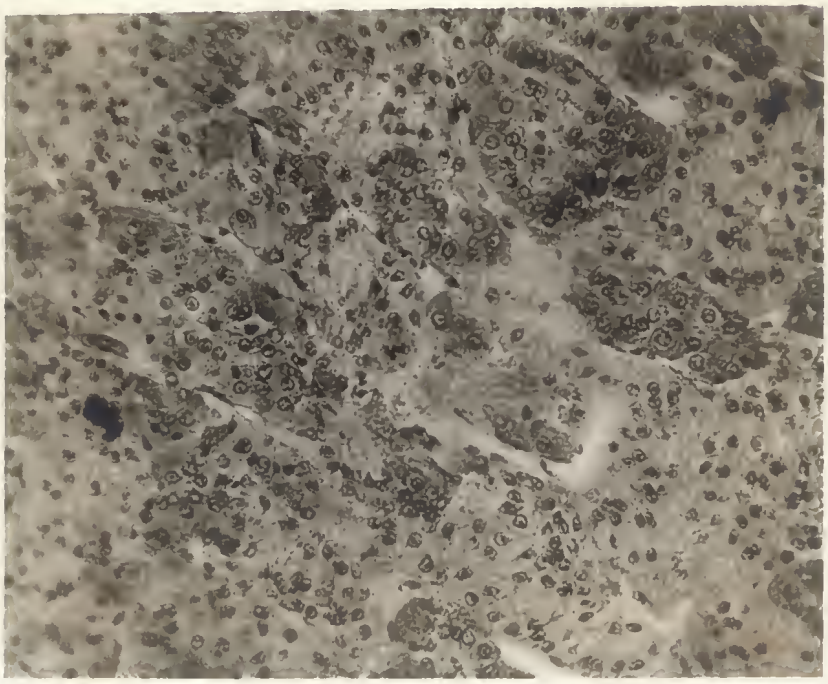

Figure 2.

Explanation of Plate II

Figure 3. Drawing of apparatus used in adrenalectomy.

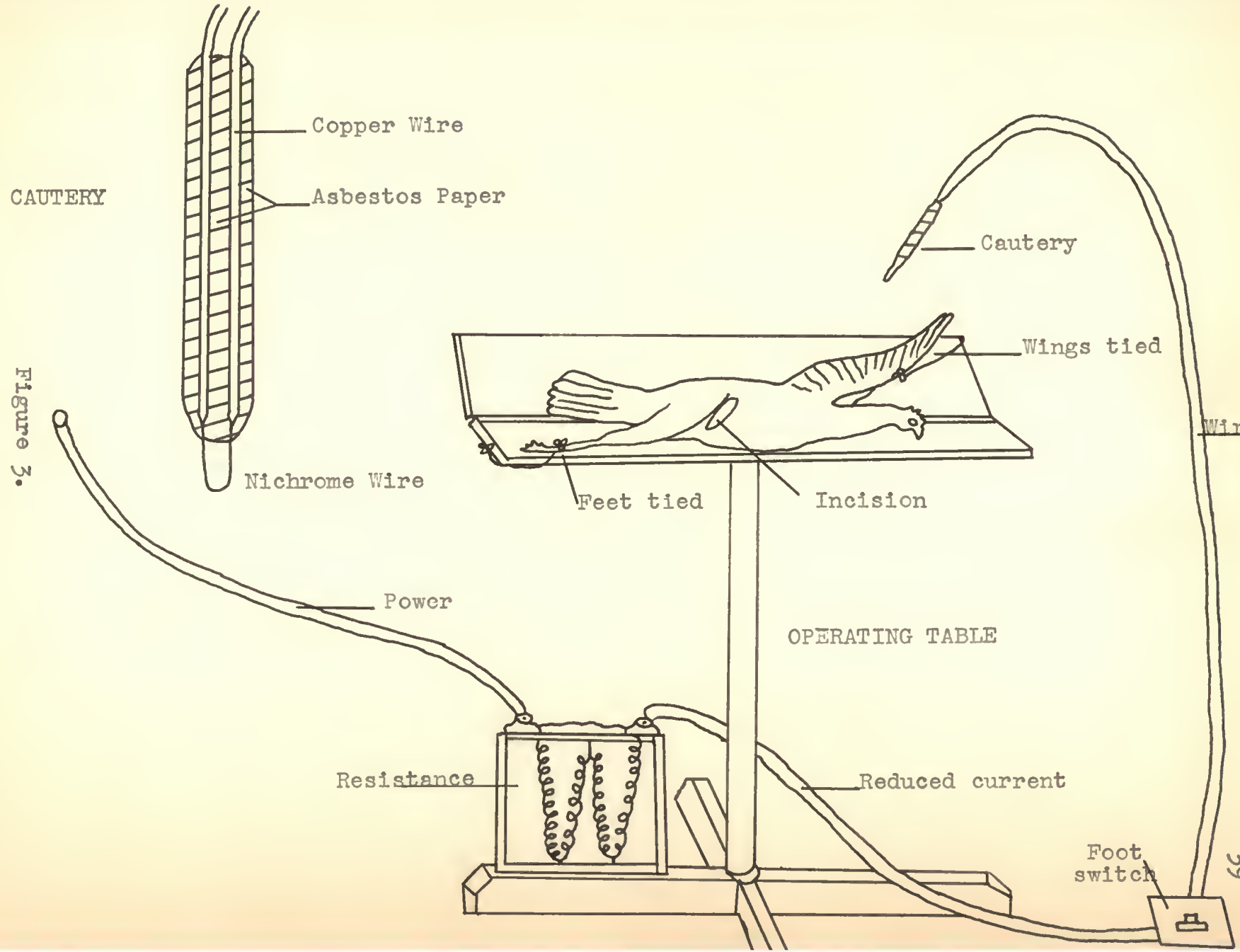

Figure 3.

Plate II.

Explanation of Plate III

Figure 4. Photograph of headdress of A300 immediately after the removal of the second adrenal gland.

Figure 5. Photograph of headdress of A300, 82 days after removal of the second adrenal gland.

Plate III

Figure 4

Figure 5

Explanation of Plate IV

Figure 6. The relative sizes of the testes of experimental and control birds.

828 Testis from sexually mature normal bird seven months old.

A 94 Testis of normal bird nine months old.

A 56 Testis of bird nine months old, 14 days after adrenalectomy.

A 58 Testis of bird nine months old, 17 days after adrenalectomy.

Plate IV.

Figure 6.

Explanation of Plate V

Figure 7. Cross section of testis of experimental bird 17 days after adrenalectomy.

Figure 8. Cross section of testis of control bird the same age as shown in figure 7.

Plate V.

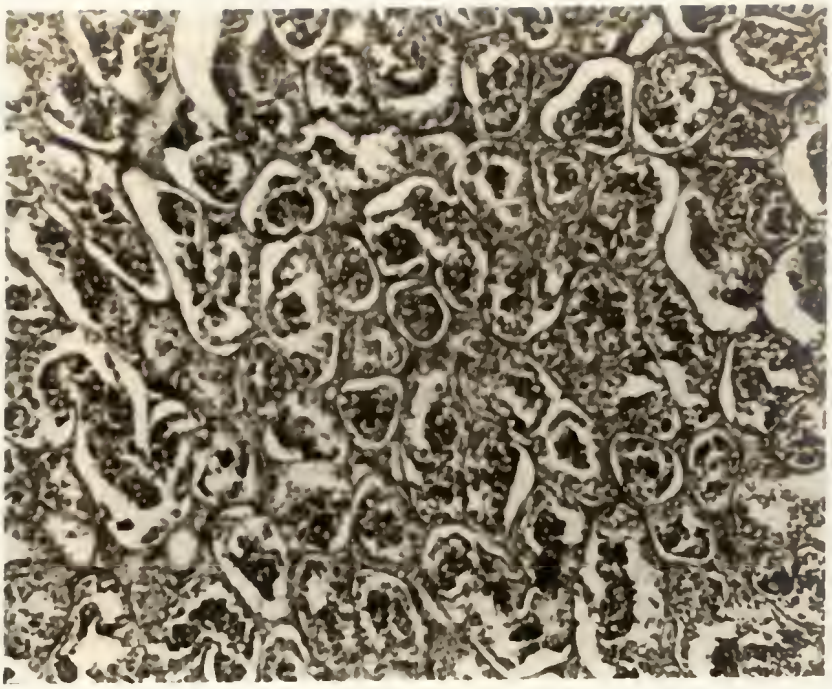

Figure 7.

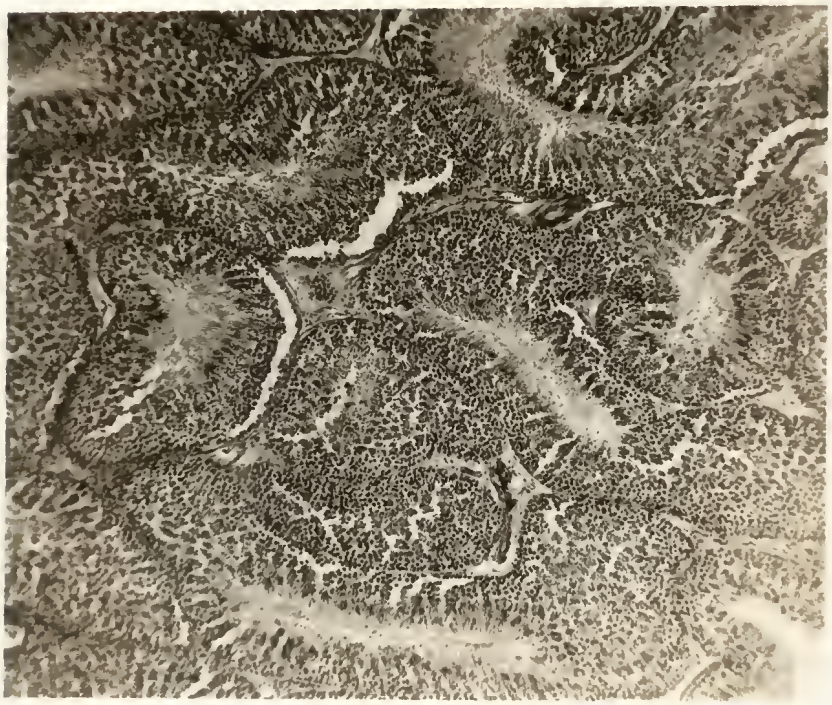

Figure 8.